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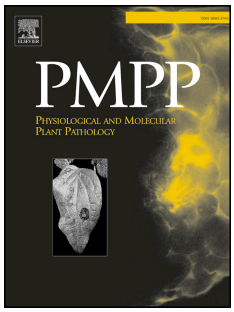

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Identification and Pathogenicity of *Fusarium* species associated with Sesame (*Sesamum indicum* L.) seeds from the Punjab, Pakistan

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ABSTRACT

Sesame (*Sesamum indicum*) is an oil-crop in Asia and Africa and is widely grown in the Punjab region of Pakistan. A total of 105 sesame seed samples were collected from different locations in the Punjab from which 520 isolates of *Fusarium* spp. were recovered. These isolates were initially grouped and identified based on morphological characteristics. The identities of representatives of the three most frequently isolated groups (strains designated F01, F98, F153) were identified as *Fusarium proliferatum*, on the basis of the sequencing of ITS of rDNA and translation elongation factor (TEF-1α) gene regions. Phylogenetic trees generated using the maximum likelihood method showed that these three isolates and a *F. proliferatum* reference sequence grouped in the same clade with *F. phyllophilum*, the most closely related species. Pathogenicity tests demonstrated that these three isolates caused disease on sesame plants. Disease Incidence (DI) and Disease Severity Index (DSI) data indicated that F01 was the most virulent isolate, with DI and DSI of approximately 70%. Culture filtrates of F01 reduced sesame seed germination (to 40%) and vigor (to 16.5%) of sesame seedlings. This baseline study suggests that *F. proliferatum* infection of sesame seeds could be a major source of yield loss in the Punjab, Pakistan which requires further attention.

Keywords: Sesame, *Fusarium*, Blotter Paper Method, ITS, TEF-1α

INTRODUCTION

Sesame (*Sesamum indicum* L.; Pedaliaceae) is one of the most important and oldest oil crops used by humans (Noorka et al. 2011). Sesame seed contains 50-60 percent oil and 22 percent protein. The oil is a semi-drying type and is mostly used in confectionary items; good quality oil is also used for medicinal purposes (Smith and Salerno 1992). A particularly useful characteristic of sesame oil is the long shelf life due to the presence of the antioxidant, sesamol (Mohamed and Awatif 1998). This quality makes it applicable for use in the manufacturing of margarine in various parts of the world where there is inadequate refrigeration. Sesame oil is also used in paints, soaps, cosmetics, perfumes, bath oils, insecticides, and pharmaceuticals (as a vehicle for drug delivery). Poppy seed, cottonseed, and rape oils are frequently added to sesame oil. Sesame seeds and young leaves are eaten as stews and used in soaps in Asia (Grubben and Denton 2004).

Sesame probably evolved in Africa and was spread early in human migrations through West Asia, China, and Japan, regions which then became secondary centers of diversity. Sesame was introduced into India by the earliest human migrants from Africa. Charred remains of sesame at Harappa excavations (3600-1750 B.C) indicate that sesame was cultivated by the Indus Valley civilization (Ashri 2007). Today, sesame (locally called 'til') is grown in 70 countries of the world, including 26 and 24 countries in Africa and Asia, respectively, with average global production close to 3 million tons per annum. The top five producers account for approximately 70% of global production. Sesame is typically grown in dry tropical and subtropical regions. In Pakistan, sesame is grown in 65 districts as an irrigated or rain-fed crop, particularly in the Punjab (Amjad 2014). Crucially, Pakistan represents a typical climatic growing area for sesame and, therefore, yield constraints in that country are relevant to other sesame producing regions (Fitzpatrick and Rene De Baaij 2013).

Diseases, particularly those caused by fungi, are major yield constraints in both sesame seed production and in seed storage (Mbah and Akueshi 2000, 2001). Sesame production faces numerous problems such as wilt, root rot and damping off, all due to attack by soil-borne fungi. In Pakistan, charcoal rot, *Alternaria* leaf blight, bacterial leaf spot, bacterial blight, phyllody, and wilting are major diseases affecting this crop. The most common and destructive diseases are wilts caused by several species of *Fusarium* and *Verticillium* (Thomson and Ockey, 1993). Infection of *Fusarium* species may also result in the accumulation of toxic secondary metabolites in the seed, damping-off of seedlings and even the death of the whole plant at the time of flowering, and thereby reduce yields (Farhan et al. 2010; Salleh and Mushitah 1991).

Accurate detection and diagnosis in the genus *Fusarium*, however, is complicated due to lack of an accurate taxonomy. A number of factors, particularly a lack of clear morphological characters separating species, had led to the broad species definitions, which, together with observed variations and mutations in culture, have led to taxonomic systems that poorly reflect species diversity. A result of this confusion, there is an inconsistent application of species names to toxigenic and pathogenic isolates (Taylor et al. 2000). Accurate methods for detecting infections of sesame seeds are required to develop robust pathogen and disease management strategies.

Monitoring of plant pathogenic microorganisms can be carried out qualitatively by following disease symptoms appearing on infected plants, or more quantitatively using molecular methods, for example, PCR amplification and sequencing of loci that are indicative of the fungal species; often referred to as the “barcode”. In this current research, we sought to apply molecular barcoding approaches to identify *Fusarium* spp. isolated from infected sesame seeds. The aim of the work was to determine the prevalence of *Fusarium* species associated

with sesame seeds in the Punjab province. Accurate identification of *Fusarium* spp. using molecular techniques and pathogenicity tests will aid in the development of improved disease management methods.

MATERIALS AND METHODS

Pathogen Isolation from Seeds and Identification

A total of 105 samples of sesame seeds were collected from the major sesame producing areas of the Punjab, Pakistan, as indicated in Figure 1. Seeds were brought to the laboratory and surface sterilized with NaOCl for 2 min. Surface sterilized and unsterilized seeds from each sample were placed on three layers of moistened filter paper (Whatman™ 1001-090 Grade 1) discs, with 25 seeds per Petri dish. The Petri dishes were incubated at 22 ± 2 ºC for seven days in an alternating cycle of light and darkness (12 hours each) in a Versatile Environmental Test Chamber (Sanyo, Japan) with illumination provided by 55 W fluorescent tubes, giving a light intensity of 125-130 µmol m^{-2} s^{-1}. The experiment was performed in triplicate. After incubation, fungal colonies emerging from the seeds were counted and isolated on potato dextrose agar (PDA; Oxoid, UK). The morphological characters were noted and isolation frequency (Fr) and relative density (RD) of fungi were recorded as follows:

$$Fr\ (\%) = \frac{ns}{N} \times 100 \qquad RD\ (\%) = \frac{ni}{Ni} \times 100$$

Where, ns is the number of samples on which a fungus occurred; N is the total number of samples; ni is the number of isolates of a given fungal genus/species; and Ni is the total number of fungal isolates obtained.

Isolates were identified on the basis of morphological characteristics with reference to published keys (Booth 1977; Domsch et al. 1980; Leslie and Summerell 2006).

DNA extraction, PCR, and DNA Sequencing

Genomic DNA was extracted using the phenol-chloroform extraction method (González-Mendoza et al. 2010). For DNA extraction, fresh cultures of *Fusarium* species were prepared by using dilution method (Cha et al. 2007). After 7 days of incubation, 50 mg mycelium of each isolate was harvested using a sterile surgical blade, and ground in liquid nitrogen using a mortar and pestle, and transferred to 1.5 ml microcentrifuge tubes. The resulting powder was suspended in 500 µl phenol and 1000 µl extraction buffer (1% sodium dodecyl sulphate, 1 M Trizma base, 100 mM NaCl, 10 mM Na_2EDTA, pH 8.0), vortexed and centrifuged at 10,000 rpm for 10 min. The supernatant was transferred to a new microcentrifuge tube and 500 µl chilled isopropanol and 50 µl 3 M, chilled sodium acetate (pH 4.8) added. Following gentle mixing, the preparation was centrifuged at 10,000 rpm for 10 min. The supernatant was discarded and the pellet was washed twice with 500 µl 70% ethanol with centrifuging, before final re-suspension in 200 µl of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0).

For molecular identification, the internal transcribed spacer (ITS) and translation elongation factor (TEF-1α) regions were amplified, based on ITS1 / ITS4 primers and EF1-983F / EF1-2212R primers, respectively (Table 1). PCR was conducted in a 25 µl reaction mixture containing 2.5 µl PCR buffer (200 mM Tris HCl (pH 8.4), 500 mM KCl), 0.5 µl dNTPs, 1.5 µl $MgCl_2$, 0.5 µl DNA polymerase, 2.5 µl each primer, 14 µlDEPC H_2O and 1 µl template DNA. The PCR reaction was performed in a MyCycler™ Thermal cycler (Bio-Rad, USA) with initial denaturation at 95 ºC for 5 min followed by 35 cycles of 95 ºC for 30s, annealing at 64 ºC for 1 min (for ITS primers) and 72 ºC for 1 min, and a final elongation step at 72 ºC for 1 min. For the TEF-1α primers, a touchdown PCR was run with an annealing temperature of 66 ºC in the first cycle, successively reducing the temperature by 1 ºC per cycle over the next 9 cycles to reach a final temperature of 56 ºC, which was used in the remaining 30-36

cycles. An extension time of 1 min 30 sec per cycle at 72 ºC was applied. Amplified fragments were analyzed on 1% agarose (Melford, UK) gel, purified and sequenced in both directions by Macrogen, Korea. Sequences were analyzed using Mega 7 software and blasted against the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Pathogenicity Test

Three most frequent *Fusarium* isolates were grown in PDA and conidia were harvested by flooding cultures with 2% Tween 20. The suspension was collected and filtered through two layers of sterilized cheesecloth. The spores were then adjusted to 1 x 10^6 conidia mL^{-1} by repeated hemocytometer counts (Garibaldi et al. 2004).

Sesame plants were grown in 13 × 11 cm plastic pots, with 3 plants per pot, containing sterilized potting medium (soil, sand, farmyard manure; 1:1:1 w/w/w). For inoculation, a channel, about 1 cm long and 3 cm deep, was created in the soil near each seedling and filled with 10 mL spore suspension. Controls were treated with sterile distilled water. Five replicate pots per treatment were prepared. Inoculated plants were covered with clear plastic bags for 24 h to maintain high humidity and placed in the glasshouse at 25/20 °C day/night temperature and irrigated with distilled water on alternate days.

Symptoms were recorded after 30 days of treatment. Disease symptoms on roots and stems were scored using a four-point categorical disease severity scale: 0 = no wilting symptoms, 1 = 25% wilting, 2 = 50% wilting and 3 = 100% wilting and plant death. The disease severity index (DSI) was calculated as ∑ (disease severity scale points × number of plants at each scale point)/ (total number of seedlings assessed × disease severity scale of the highest scale point observed) × 100 (Zhao et al. 2014).

To verify that the inoculated *Fusarium* isolates caused the symptoms on the sesame seedlings, re-isolations were made from symptomatic parts of inoculated plants. After seven days of inoculation, symptomatic tissues were surface sterilized in 5% NaOCl twice for 2 min followed by rinsing with autoclaved distilled water for 2 min. Three pieces (3-6 mm long) of roots or stems per plant were excised under aseptic conditions and transferred to PDA in Petri dishes. Dishes were incubated at 28ºC until the appearance of typical *Fusarium* colonies, approx. 6 days after plating.

Effect of Culture Filtrate on Seed Germination and Seedling Growth

Erlenmeyer flasks containing 50 ml of potato dextrose broth were inoculated with the test fungi (1 x 10^6 conidia mL^{-1}) and incubated for 14 days at 28 °C. Then, the culture broth and fungal mycelia were carefully separated. A 50 ml volume of 3:2:1 (v/v/v) ethyl acetate: chloroform: methanol was added to each flask containing culture broth, followed by shaking overnight on a rotary shaker. Extracts were centrifuged at 5000 rpm for 30 min and the supernatant was incubated in a water bath at 45 °C for 8-10 h to concentrate the extract to a volume of 10 ml (Jaiswal et al. 2012).

Sesame seeds were surface sterilized in 5% NaOCl for 2 min followed by 3 rinses in sterile distilled water and then suspended in culture filtrates (10 ml). Following incubation at 28 ± 2ºC for 24 h, seeds were removed from the culture filtrates and washed in sterile distilled water. Treated seeds were plated on 1.5 % water agar, with 10 seeds per Petri dish. Control seeds were treated with distilled water. After 7 days of incubation, shoot and root lengths were recorded. In addition, a vigor index was calculated (Jalander and Gachande 2012) following the formulae:

Germination % = Germinated seeds of treatment/Germinated seeds of control x 100

Vigor index = Seed germination (%) × Seedling Length (Shoot + Root Length)

Statistical Analysis

Analysis of variance (ANOVA) was performed on the data of DI, DSI, germination percent, and vigor index to determine the effects of representative *Fusarium* isolates on sesame seeds and seedlings. The mean differences were compared by Duncan's multiple range test ($P < 0.05$). All calculations were made using SPSS (version 16.0; SPSS Inc, Chicago, IL).

RESULTS

Morphological characterization of *Fusarium* isolates

A total of 520 isolates of *Fusarium* were recovered from sesame seeds, separated into 23 morphological groups and identified on the basis of colony characters and conidial structure. Three isolates (F01, F98, F153) as representative of the most frequent morphological groups were identified as *F. sacchari* (39%), *F. phyllophilum* (40%) and *F. culmorum* (30%), respectively (Table 2). Initially, these isolates had hyaline aerial mycelium which turned to white or purple as colonies aged, and various pigmentations from white to light purple, through deep reddish purple to dark reddish purple were observed. The growth rate ranged 4-8 cm in 7 days. Most macroconidia were slender with a curved apical cell and a notched basal cell. They were 3-5 septate with size ranged as 25-55 x 3-5µm. Microconidia from all isolates were 0- septate, 7-12 x 2.2-3.0µm in size, oval with flattened bases and were mostly formed in a false head (small, mucoid, adherent balls of conidia) (Figure 2). However, chlamydospores were not found in any of these cultures. All the identified isolates were submitted in the First Fungal Culture Bank of Pakistan (FCBP), University of the Punjab,

Lahore, Pakistan. These three representative isolates were selected for molecular characterization and pathogenicity tests.

Molecular characterization of *Fusarium* Isolates

Selected isolates were identified using the ITS region of rDNA and TEF-1α gene. Separation of the PCR products of ITS amplification on an agarose gel revealed amplicons of ~650 bp in length. All sequences exhibited 100% identity with *Fusarium proliferatum* ITS sequences in GenBank (Table 3) (Figure 3). Analysis of the TEF-1α sequences showed > 90% sequence identity to *Fusarium proliferatum* although, some sequence variation between isolates was observed (Table 3; Figure 4). Phylogenetic analysis suggested that F98, compared to F01 and F153, was most closely related to the *Fusarium proliferatum* sequences present in GenBank. TEF-1α sequences from all three isolates, however, fall in the same clade.

Pathogenicity Tests

Inoculation of sesame plants with isolates F01, F98 and F153 resulted in the browning of foliage by approximately 14 days after treatment, after which plants continued to decline, becoming dark brown and necrotic. Roots became semi-transparent, shrunken, water-soaked and eventually disintegrated and whole plants wilted after 30 days (Figure 5). The statistical data revealed that there was a significant difference ($P<0.05$) among the treatments. Although, the mean difference was non-significant ($P>0.05$) between F01 and F98 for disease incidence but the results of DSI showed a significant difference ($P<0.05$) among three isolates. Hence F01 (70) was considered more pathogenic than F98 (53.33) and F153 (23.33) based on disease severity assessments (Table 4). In control plants, 100% green leaves were maintained over the assessment period. Re-isolations of the same three isolates were

successful: all re-isolated strains were morphologically identical to the fungus used in the inoculations of the sesame plants.

Effect of *Fusarium* Culture Filtrates on Germination of Sesame

All three isolates showed the highly significant difference ($P<0.05$) as compared to control. Seed germination and vigor of sesame plants were adversely affected by culture filtrates of isolate F01, with a 40-66% reduction in seed germination, and a 16.5% reduction in vigor, when compared with control plants (Table 5; Figure 6). Germination rates of 66 and 63% occurred in seeds treated with isolates F153 and F98, respectively. Overall, F01 showed a highly significant reduction in germination percentage.

DISCUSSION

Fusarium is a major cause of wilt in sesame but the range of species causing the disease in this host remains poorly defined. In this study, we surveyed the major sesame growing areas of the Punjab (Pakistan) for wilt symptoms in order to determine the *Fusarium* species causing the disease. Problems using morphological characteristics alone to identify *Fusarium* species have been reported frequently (O'Donnell et al. 1998, 2000; Marasas et al. 2001; Steenkamp et al. 1999). However, initial identification and characterization using morphological features is important to separate isolates into smaller groups before applying other methods of identification (Leslie and Summerell 2006). Distinguishing species within the genus *Fusarium* using morphological characters is difficult even for specialists (Summerell et al. 2003; Leslie and Summerell 2006) and it is now accepted that DNA sequence-based identifications and PCR assays are needed to accurately identify species within the complex genus *Fusarium*. The three most frequently isolated *Fusarium* species

identified initially as *F. sacchari* (39%), *F. phyllophilum* (40%) and *F. culmorum* (30%), therefore, were subjected to "DNA bar-coding" approaches to obtain more stringent identifications.

PCR and sequencing of the ITS regions have become a routine method for the detection, identification, classification and phylogenetic analysis of many fungi at the species level (Taylor et al. 2000). Sequences of the ITS regions can distinguish *Fusarium* from other fungi, and taxon-selective ITS amplification has been used to detect *Fusarium* spp. (e.g. Pearson et al. 2016; O'Donnell 1992). In the present work, ITS sequencing confirmed isolates obtained from sesame were *Fusarium* species, but further discrimination required the use of translation elongation factor-based primers. Translational elongation factor 1α is a highly conserved protein encoding region, which can be used to resolve between closely related species, and has proved useful for phylogenetic and taxonomic analyses of *Fusarium* spp. (Geiser et al. 2004). Use of TEF-1α primers demonstrated that the three *Fusarium* isolates tested here were all *F. proliferatum*, providing a platform on to which to define virulence mechanisms in sesame.

Previous reports on the occurrence and pathogenicity of *Fusarium* spp. were solely based on samples isolated from infected, symptomatic plants. For example, various *Fusarium* species including *F. oxysporum, F. proliferatum* and *F. redolens* proved highly virulent on onion, causing the death of many inoculated seedlings. Among the isolates tested, *F. proliferatum* proved highly pathogenic to onion in inoculation tests (Haapalainen et al. 2016). It is likely that *F. proliferatum* is a problematic pathogen on a range of host plants, as this fungal species is also reported causing serious problems on corn and wheat as well as onion (Conner et al. 1996; Logrieco et al. 2002).

There is little information available in the literature on pathogens attacking sesame during germination. Work in Pakistan demonstrated that culture filtrates of *Xanthomonas campestris* pv. *sesami* (Xcs) reduced germination progressively with increasing concentrations of the filtrates (Firdous et al. 2013). According to another study conducted in the Punjab (Pakistan), seeds and seedlings health of sesame were greatly affected by the spore suspension and culture filtrates of *Alternaria alternata* (Nayyar et al. 2017). In the present work, both inoculations with spore suspension and treatment with culture filtrates of *Fusarium* isolate F01 suppressed germination and growth of sesame. It was assumed that toxic metabolites produced into the culture filtrates by *Fusarium* were responsible for these symptoms. Toxins produced by *Fusarium oxysporum* f. sp. *ciceris* (FOC) were shown to affect root growth in chickpea and were thought to impact negatively on seed germination of chickpea (Khan et al. 2004). Deoxynivalenol, T-2 toxin, fumonisin B1, and nivalenol produced by *Fusarium* sp. are known to suppress seed germination (Zonno and Vurro 1999). The production of secondary metabolites by fungi is known to reduce seed quality and viability (Gopinath and Shetty 1988). Similar results were also observed on seeds of pigeon pea, chickpea and tomato varieties (Raithak and Gachande 2013; Arya and Mathew 1991).

CONCLUSION

The most frequent *Fusarium* isolates obtained from sesame seed collected in the Punjab, Pakistan were identified as *F. proliferatum* on the basis of molecular analyses. One isolate of *F. proliferatum*, F01, was more virulent than the other two isolates tested, causing damping-off, and reductions in growth and vigor of sesame seedlings.

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Table 1. Primers used for molecular identification of selected *Fusarium* isolates

Code	Primer Sequence (5'-3')	Target	Reference
ITS 1 (F)	TCCGTAGGTGAACCTGCGG	18S rDNA	White et al. (1990)
ITS 4 (R)	TCCTCCGCTTATTGATATGC	28S rDNA	White et al. (1990)
EF1-983 (F)	GCYCCYGGHCAYCGTGAYTTYAT	TEF-1α	Rehner and Buckley (2005)
EF1-2212 (R)	CCRACRGCRACRGTYYGTCTCAT	TEF-1α	Rehner and Buckley (2005)

Table 2. Morphological characterization, isolation frequency and relative density of *Fusarium* isolates in sesame seeds

Isolate Code	Name of Fungi	Origin (city)	Surface Non Sterilized Seeds			Surface Sterilized Seeds			FCBP accession no.
			No. of isolates	*Fr*	*RD*	No. of isolates	*Fr*	*RD*	
F01	*Fusarium sacchari*	Sialkot	63	39	16.45	18	12	13.14	1416
F04	*Fusarium oxysporum*	Sialkot	1	2	0.26	0	0	0.00	1432
F12	*Fusarium torulosum*	Sialkot	9	12	2.35	0	0	0.00	1424
F14	*Fusarium phyllophilum*	Sialkot	11	22	2.87	8	12	5.84	1423
F15	*Fusarium oxysporum*	Sialkot	13	16	3.39	5	12	3.65	1442
F22	*Fusarium* sp.	Gujranwala	3	2	0.78	0	0	0.00	1428
F27	*Fusarium subglutinans*	Gujranwala	4	14	1.04	4	12	2.92	1444
F30	*Fusarium polyphialidicum*	Gujranwala	6	14	1.57	4	12	2.92	1445
F31	*Fusarium subglutinans*	Gujranwala	5	0	1.31	9	8	6.57	1446
F40	*Fusarium sacchari*	Gujranwala	1	2	0.26	1	2	0.73	1462
F52	*Fusarium oxysporum*	Gujranwala	12	0	3.13	10	2	7.30	1458
F74	*Fusarium solani*	Gujranwala	3	6	0.78	0	0	0.00	1460
F80	*Fusarium culmorum*	Gujranwala	12	4	3.13	2	0	1.46	1459
F84	*Fusarium lateritium*	Gujranwala	26	2	6.79	0	0	0.00	1455
F98	*Fusarium phyllophilum*	Gujranwala	55	40	14.36	12	26	8.76	1441
F153	*Fusarium culmorum*	Gujranwala	66	30	17.23	43	12	31.39	1418
F174	*Fusarium venenatum*	Gujranwala	26	26	6.79	10	4	7.30	1471
F190	*Fusarium redolens*	Gujranwala	37	4	9.66	0	2	0.00	1469
F269	*Fusarium semitectum*	Mandi Bahuddin	11	2	2.87	2	0	1.46	1464
F286	*Fusarium semitectum*	Mandi Bahuddin	14	2	3.66	2	2	1.46	1464
F311	*Fusarium scirpi*	Chakwal	4	2	1.04	5	2	3.65	1472
F346	*Fusarium equiseti*	Bahawalnagar	0	2	0.00	1	2	0.73	1468
F349	*Fusarium* sp.	Bahawalnagar	1	2	0.26	1	2	0.73	1473

Fr= Isolation frequency; RD= Relative Density; FCBP= Fungal Culture Bank of Pakistan

Table 3. Molecular Identification of three most frequent *Fusarium* isolates from sesame seeds.

Isolate Code	Origin (city)	Morphological Identification	Molecular Identification	NCBI accession no.	
				ITS	TEF-1α
F01	Sialkot	*Fusarium sacchari*	*Fusarium proliferatum*	KX901460	KY247083
F98	Gujranwala	*Fusarium phyllophilum*	*Fusarium proliferatum*	KX901461	KY247084
F153	Gujranwala	*Fusarium culmorum*	*Fusarium proliferatum*	KX901462	KY247085

Table 4. Effects of spore suspension of three *Fusarium* isolates on disease incidence and disease severity in sesame seedlings.

Treatment	Total Seedlings	Wilted seedlings	Disease Incidence (%)	Disease Severity (DSI)	Rating Scale
F01	10	7	70b	70.00c	3
F98	10	8	80b	53.33b	2
F153	10	3	30a	23.33a	1

Values with same letter are not significantly different based on Duncan's multiple range test (P<0.05).

Table 5. Effects of culture filtrates of *Fusarium* isolates on seed germination and seedling growth of sesame.

Treatment	Germination %	Root length (cm)	Shoot Length (cm)	Vigour Index
F01	40.00a	0.40a	0.50a	16.50a
F98	63.33b	0.40a	0.80a	26.13a
F153	66.67b	0.40a	1.00ab	27.67a
Control	86.67c	0.70b	1.50b	62.17b

Values with same letter are not significantly different based on Duncan's multiple range test ($P<0.05$)

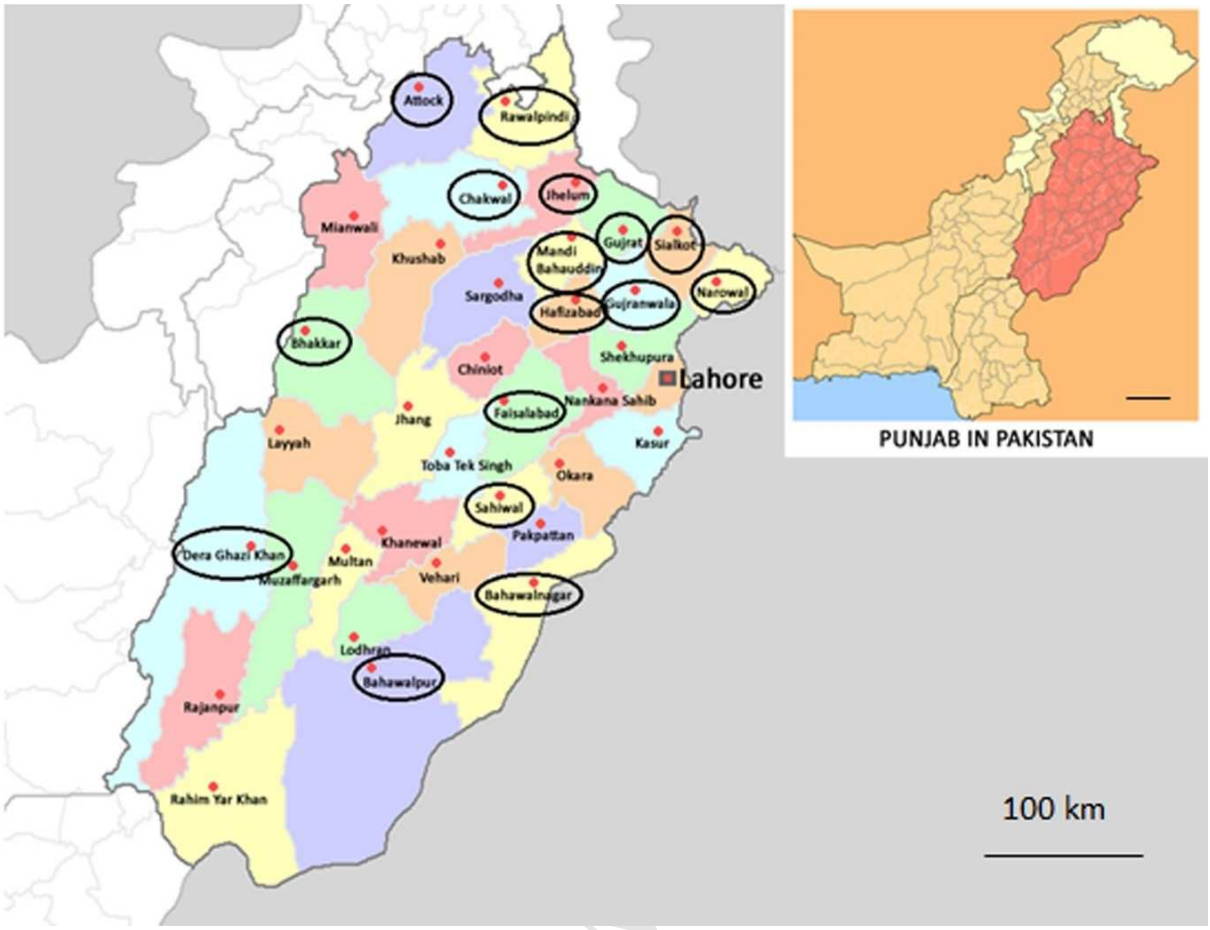

Figure 1. Map of the Punjab (Pakistan) indicating the major sesame growing districts where the sesame seeds were collected.

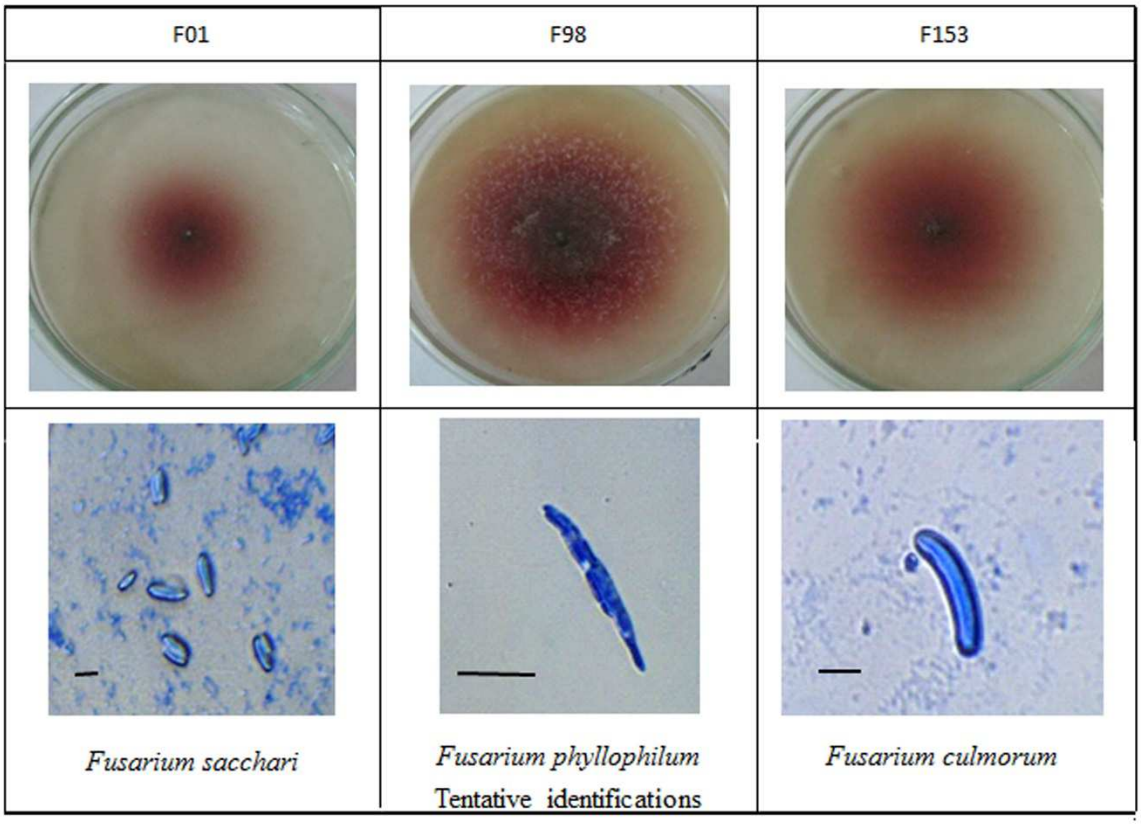

Figure 2. Colony and microscopic characters of three most frequent representative *Fusarium* isolates (F01, F98, F153) isolated from sesame seeds (bar = 20 µm).

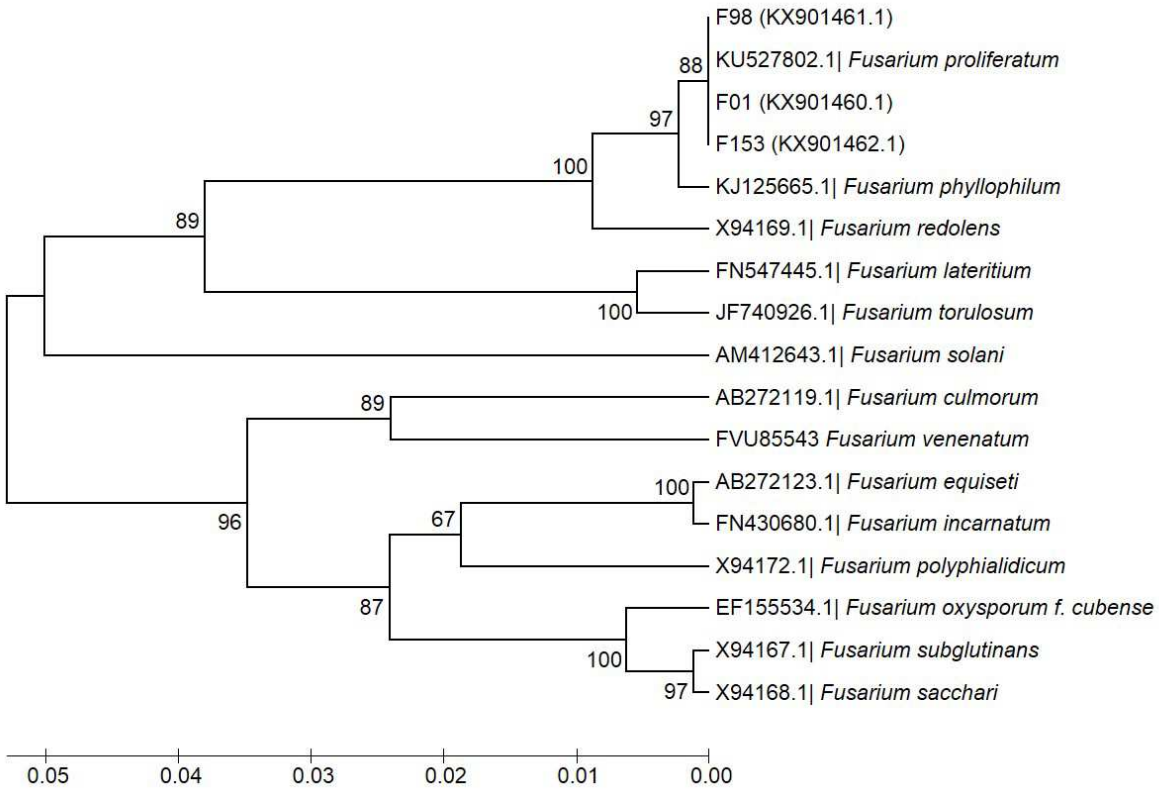

Figure 3. Maximum likelihood phylogenetic tree obtained from consensus sequences of *Fusarium* isolates (F01, F98, F153) from sesame seeds using ITS primers. Numbers above the branches are bootstrap values from 500 replicates. The scale indicates the genetic distance between the species.

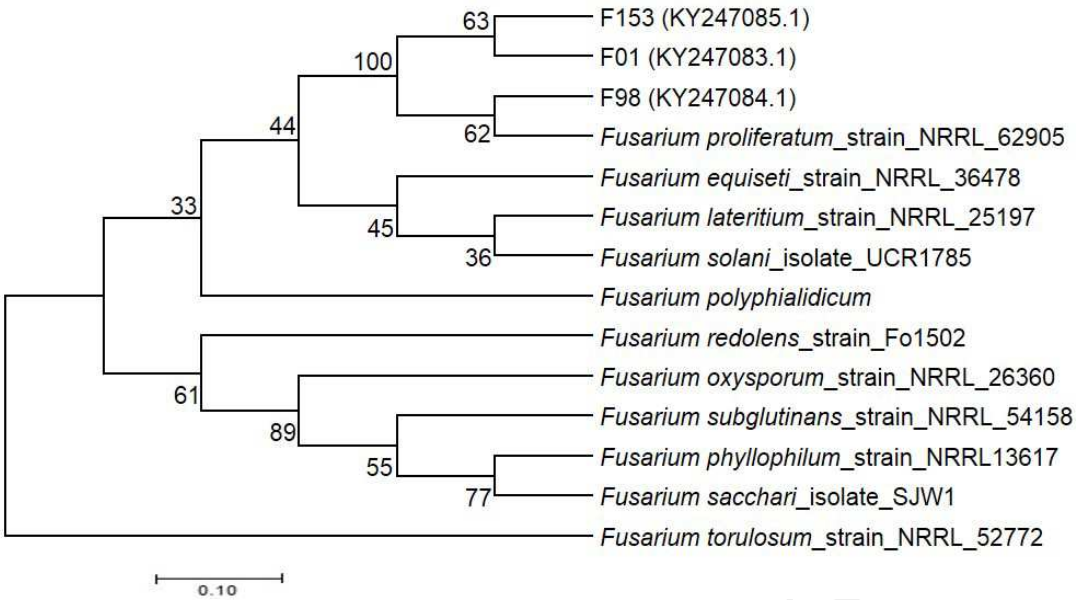

Figure 4. Maximum likelihood phylogenetic tree obtained from consensus sequences of *Fusarium* isolates (F01, F98, F153) from sesame seeds using translation elongation factor (TEF-1α) primers. Numbers above the branches are bootstrap values from 500 replicates. The scale indicates the genetic distance between the species.

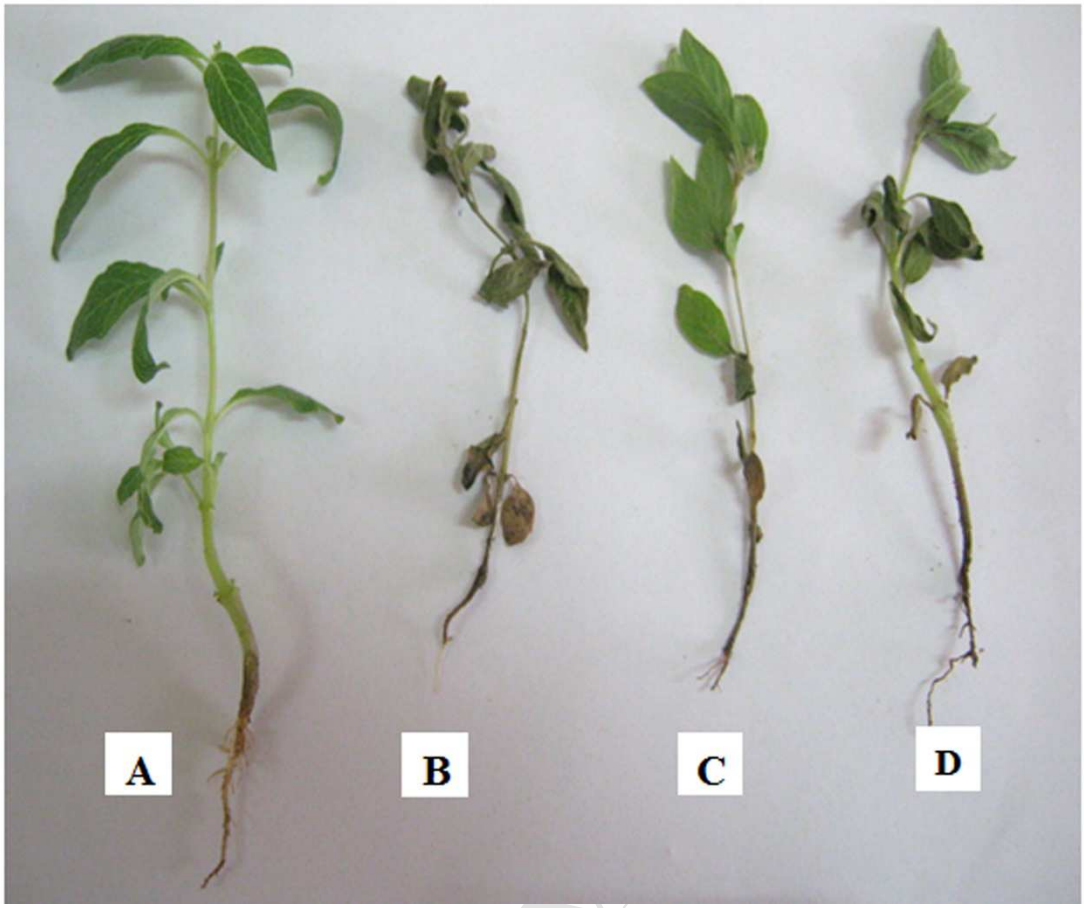

Figure 5. Thirty days post inoculation symptoms of three *Fusarium* isolates on sesame plants. (A) Control; (B) F01; (C) F98; (D) F153

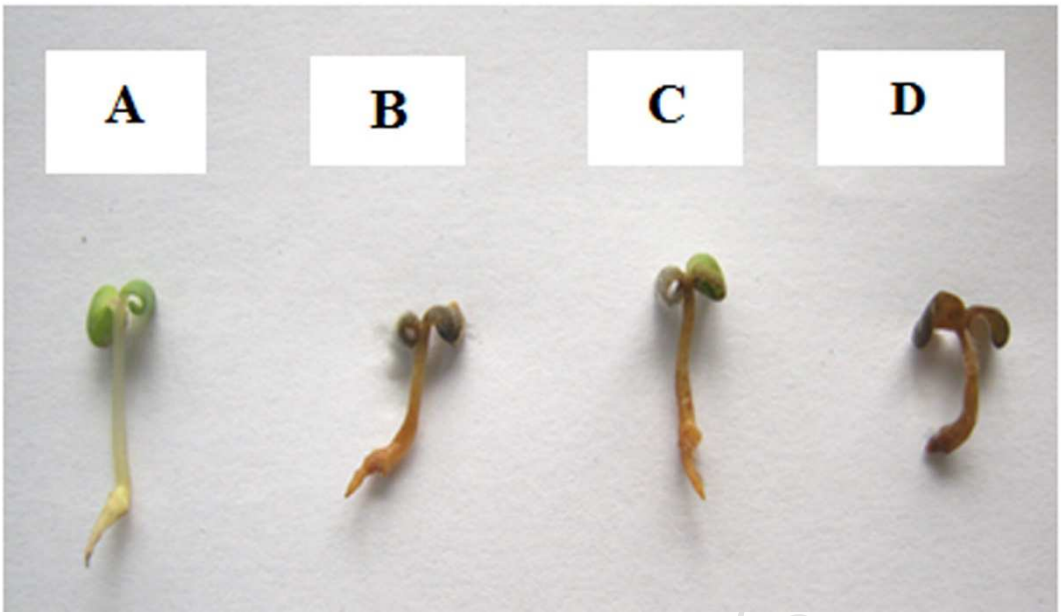

Figure 6. Seven days post treatment effects of culture filtrates of *Fusarium* isolates on germination of sesame seeds. (A) Control; (B) F01; (C) F98; (D) F153

Identification and Pathogenicity of *Fusarium* species associated with Sesame (*Sesamum indicum* L.) seeds from Punjab, Pakistan

Highlights

- Sesame yielded *Fusarium* species as dominant pathogen.
- Three most frequent isolates were accurately identified as *Fusarium proliferatum* through DNA barcoding.
- These isolates were found as virulent with 70% disease severity index and reduced seed germination and vigor index of sesame plants.
- It can be suggested that *Fusarium proliferatum* infection might be a major source of sesame yield loss in the Punjab, Pakistan which requires further confirmation.